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Specific Detection of *Campylobacter jejuni* and *Campylobacter coli* by Using Polymerase Chain Reaction

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Development of a routine detection assay for *Campylobacter jejuni* and *Campylobacter coli* in clinical specimens was undertaken by using the polymerase chain reaction (PCR). An oligonucleotide primer pair from a conserved 5′ region of the *flaA* gene of *C. coli* VC167 was used to amplify a 450-bp region by PCR. The primer pair specifically detected 4 strains of *C. coli* and 47 strains of *C. jejuni*; but it did not detect strains of *Campylobacter fetus*, *Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter cryaerophila*, *Campylobacter butzleri*, *Campylobacter hyointestinalis*, *Wolinella recta*, *Helicobacter pylori*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, *Citrobacter freundii*, or *Aeromonas* spp. By using a nonradioactively labeled probe internal to the PCR product, the assay could detect as little as 0.0062 pg of purified *C. coli* DNA, or the equivalent of four bacteria. In stools seeded with *C. coli* cells, the probe could detect between 30 and 60 bacteria per PCR assay. The assay was also successfully used to detect *C. coli* in rectal swab specimens from experimentally infected rabbits and *C. jejuni* in human stool samples.

The thermophilic *Campylobacter* species, particularly *C. jejuni* and *C. coli*, are among the most frequently isolated bacteria that cause diarrheal disease in humans (34, 36). These microaerophilic organisms are more fastidious and slower growing than other bacterial enteropathogens. The methods used for clinical isolation of campylobacters vary considerably among laboratories, but all methods require special growth conditions and, often, specialized transport media to ensure that these oxygen-sensitive organisms remain viable prior to plating. Such problems with isolation often result in an inability to recover and identify the organisms. This is of special concern during epidemiological investigations in which large numbers of a variety of different sample types may need to be analyzed rapidly to determine the source of an outbreak.

The polymerase chain reaction (PCR) has been applied extensively to the detection of infectious agents (8). PCR allows amplification of a preselected region of DNA and can be a highly specific and sensitive detection technique (24). PCR has also been used for the direct identification of organisms from complex substrates without prior isolation and purification of the organisms (26, 27, 33, 41, 42). The difficulties in routine detection, isolation, and identification of *Campylobacter* spp. make these organisms ideal candidates for PCR identification. Moreover, the phylogenetic distance of *Campylobacter* spp. from other enteric pathogens (30) suggests that it should be relatively easy to identify campylobacter-specific target genes by PCR. One *Campylobacter* gene which has the potential to allow for organism identification at the level of species and at the narrower level of strain is the flagellin gene. The flagella of *C. jejuni* and *C. coli* are composed of two subunit flagellins, the products of the *flaA* and *flaB* genes (9, 13, 25). We have previously cloned and sequenced these two flagellin genes from *C. coli* (13, 15, 21) and have shown that they contain highly variable regions which could be used for strain-specific detection and other regions which are highly conserved among *C. coli* and *C. jejuni* strains (37). Therefore, these genes are potentially useful for the detection of the two *Campylobacter* species most commonly associated with human diarrheal disease. In the study described here we evaluated the use of a conserved portion of the campylobacter flagellin gene as a target for PCR identification of these thermophilic enteropathogenic campylobacters and present evidence that the technique can be successfully applied to the direct detection of these organisms in human fecal material.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All bacterial strains used in this study and their sources are listed in Table 1. *Campylobacter* and *Helicobacter* spp. were grown on Mueller-Hinton medium in an atmosphere of 10% CO_2–5% O_2–85% N_2 at 37°C. Members of the family *Enterobacteriaceae* were grown on Luria agar (22). Other bacteria were grown on blood agar plates (Remel, Lenexa, Kans.) and were incubated under normal atmospheric conditions at 37°C; *Wolinella recta* was grown anaerobically, however.

DNA extractions. DNA extractions for the PCR assay were done by three different protocols. Purified DNA from campylobacters was prepared as described previously (16, 21). Two different protocols for the extraction of crude DNA from bacterial cultures were used: the method of Frankel et al. (10), which involves a phenol-chloroform extraction, and the boiling method of van Eys et al. (40). Extraction of DNA from rectal swab specimens from rabbits was performed by the method of Frankel et al. (10). Plasmid DNAs were isolated as described previously (1).

DNA primers and PCR amplification. Oligonucleotides pg50 (5′-ATGGGATTTCGTATTAAC-3′) and pg3 (5′-GAA CTTGAACCGATTTG-3′) were synthesized by Synthecell, Gaithersburg, Md. Their positions within the two tandemly oriented flagellin genes of VC167 are shown in Fig. 1. These

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TABLE 1. Bacterial strains tested with primer set pg3-pg50

Organism	Total no. of strains tested	Strain or site of isolation (no. of strains)	Source[a]
C. coli	4	VC167	Univ. Victoria
		Canada	Univ. Victoria
C. jejuni	47	United States (2)	NMRI
		Canada (6)	Univ. Victoria
		Yemen (9)	NAMRU3
		Egypt (5)	NAMRU3
		West Africa (4)	NAMRU3
		Peru (4)	NAMRID
		Panama (1)	NMRI
		Mexico (1)	NMRI
		Indonesia (15)	NAMRU2
C. lari	3	D67	CDC
		D110	CDC
		D382	CDC
C. butzleri	1	D2676	CDC
C. cryaerophila (Arcobacter cryoaerophilus)	1	D2792 (type strain)	CDC
C. hyointestinalis	3	D2189	CDC
		D2411 (porcine)	CDC
		D1932 (type strain)	CDC
C. upsaliensis	1	D1673	CDC
C. fetus subsp. *intermedius*	3	Australia	Univ. Victoria
C. fetus subsp. *fetus*	7	Canada	Univ. Victoria
W. recta (*C. recta*)	1	D2083	CDC
H. pylori	4	Canada	Univ. Victoria
V. cholerae O1	11	Kuwait or Iraq (7)	NAMRU3
		Senegal (1)	CVD
		Kenya (2)	CVD
		United States (1)	CVD
V. cholerae non-O1	2	United States (1)	CVD
		Mexico (1)	CVD
A. hydrophila	10	Canada	Univ. Victoria
A. sobria	1	A412	Univ. Victoria
A. salmonicida	8	Canada	Univ. Victoria
E. coli K-12	1	DH5α	BRL
Enterotoxigenic *E. coli*	52	Egypt (30)	NAMRU3
		Saudi Arabia (18)	NAMRU3
		263 (1)	CVD
		Throop (1)	CVD
		286 (1)	CVD
		SA53 (1)	CVD
C. freundii	1	United States	WRAIR
S. dysenteriae	3	60R (1)	WRAIR
		Egypt (2)	NAMRU3
S. flexneri	2	Egypt (1)	NAMRU3
		24570 (1)	WRAIR
S. sonnei	8	Saudi Arabia	NAMRU3
S. typhi	5	643 (1)	WRAIR
		Egypt (4)	NAMRU3
S. typhimurium	1	LT2	WRAIR
S. enteritidis	4	Saudi Arabia	NAMRU3

[a] Sources of strains were as follows: WRAIR, Walter Reed Army Institute of Research; NMRI, Naval Medical Research Institute; NAMRID, Naval Medical Research Institute Detachment, Lima, Peru; NAMRU3, Naval Medical Research Unit 3, Cairo, Egypt; NAMRU2, Naval Medical Research Unit 2, Jakarta, Indonesia, CDC, Centers for Disease Control, Atlanta, Ga.; CVD, Center for Vaccine Development, Baltimore, Md.; BRL, Bethesda Research Laboratories, Gaithersburg, Md.; and Univ. Victoria, University of Victoria strain collection.

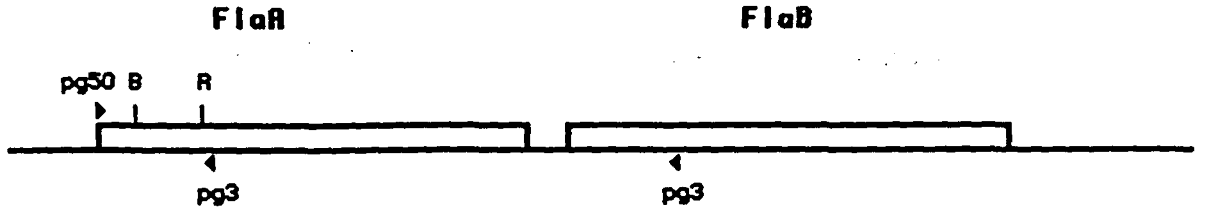

FIG. 1. Schematic representation of the two flagellin genes of *C. coli* VC167. The binding positions of primers pg50 and pg3 are indicated. Restriction sites are as follows: B, *Bgl*II; R, *Eco*RI. The region between these two restriction sites represents the 273-bp fragment present in pBA273.

oligonucleotides are located within a region of the *flaA* gene which has been shown to be highly conserved among *C. jejuni* and *C. coli* strains by DNA hybridization studies (37). The amplification reaction was performed in a volume of 100 μl containing 0.13 to 1.0 μg of sample DNA; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin; 200 μM (each) dATP, dCTP, dTTP, and dGTP; 200 ng of each primer; and 2.5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). After overlaying with mineral oil, the samples were subjected to 25 cycles of amplification in a DNA thermal cycler (Perkin-Elmer Cetus). Parameters for the amplification cycles were denaturation for 1 min at 94°C, annealing of primers for 1 min at 37°C, and primer extension for 1 min at 72°C.

Construction of DNA probe. A probe internal to the region of the flagellin gene amplified by primers pg50 and pg3 was constructed as follows. *C. coli* VC167 DNA was amplified as described above by using pg50 and pg3, and the reaction products were digested with *Bgl*II and *Eco*RI and ligated to pUC18 DNA which had been digested with *Bam*HI and *Eco*RI. Following transformation into DH5α cells, ampicillin-resistant, *lacZ* transformants were screened for the presence of a 273-bp insert into pUC18 by digesting them with *Xba*I and *Eco*RI. The purified fragment from one such clone, pBA273, was shown to hybridize to clones of the VC167 flagellin gene (data not shown). This insert from pBA273 was used as a DNA probe.

Digoxigenin-labeled probe preparation and hybridization. The 273-bp probe was labeled with digoxigenin-labeled dUTP by random priming by using the Genius kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). PCR products were transferred to Hybond membranes (Amersham Corp., Arlington Heights, Ill.) by dot blotting or Southern blotting (22) following electrophoresis in 2% agarose gels and were cross-linked with UV light (Hoefer, San Francisco, Calif.). Prehybridization was for 2 h at 60°C in 5× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with the blocking reagents recommended by Boehringer Mannheim. Hybridization was performed in the same buffer for 16 to 24 h at 60°C with 230 ng of digoxigenin-labeled probe per 100 cm^2 of membrane. Following hybridization the membranes were washed in 2× SSC–0.1% sodium dodecyl sulfate at 60°C four times for 15 min each time. The bound digoxigenin probe was detected by using the Genius kit (Boehringer).

Rabbit experiments. The rabbit experiments were conducted according to principles described previously (37a). Female New Zealand White rabbits (weight, 0.9 to 1.1 kg; Hazelton Research Products, Denver, Pa.) were fed bacterial suspensions as described previously (28). Uninfected control rabbits were fed sterile broth. Animals were held for quarantine and acclimatization in a special holding area for at least 7 days before use. Food was withheld 18 to 24 h prior to oral administration of 15 ml of a bacterial suspension (approximately 5 × 10^9 CFU/ml) with a feeding tube (2.7 mm [outer diameter] by 381 mm [length]) after neutralization of gastric acidity (6, 28). Rabbits were monitored by obtaining rectal swab specimens at approximately 48 h postinfection. The swabs were transported to the laboratory in Cary Blair transport medium (Oxoid Ltd., Basingstoke, England). The fecal material on the swabs was resuspended in 0.5 ml of phosphate-buffered saline to an optical density at 550 nm of approximately 0.35. Aliquots of this suspension were plated directly onto campylobacter blood agar plates (Remel) and were incubated microaerobically for 48 h at 37°C. Aliquots were also processed for PCR analysis by the method of Frankel et al. (10).

Human stool samples. Human stool samples, which were obtained from Naval Medical Research Unit 3, Cairo, Egypt, were clinical specimens which had been submitted for routine bacteriological analysis from patients with acute diarrhea and which had been stored frozen for 3 to 12 months. Matched bacterial isolates from *C. jejuni*-positive stools were also obtained. Stools were extracted by a modification of the method of Frankel et al. (10) as developed by Branstrom et al. (2).

RESULTS

Selection and specificities of primer pairs. Previous DNA hybridization studies that used different regions of a flagellin gene from *C. coli* VC167 as probes (37) indicated that there is a high degree of sequence variability in the central region of campylobacter flagellin genes and a high degree of sequence similarity in the 5′ and 3′ regions of the gene in the 30 strains of *C. coli* and *C. jejuni* examined (37). In addition, N-terminal amino acid sequencing of flagellins from various strains of *C. coli* and *C. jejuni* also indicated a high degree of conservation at the amino terminus of the protein (20). We therefore selected a primer pair from within the N-terminal region of the *flaA* gene, as seen in Fig. 1. Primer pg50 binds to the beginning of *flaA*, but not *flaB*; primer pg3 binds to the second strand 450 bp downstream from the pg50-binding site on *flaA* as well as at the corresponding position of *flaB* (13, 15, 21). When used in a PCR assay with VC167 DNA, the primers generate a primary product which runs at the predicted 450-bp size when the products are assayed by electrophoresis through a 2% agarose gel (data not shown). Theoretically, a second PCR product of 1.8 kb could be generated from within *flaB* by pg3 priming. However, the 1.8-kb product has not been observed, most likely because pg3 can bind at two points within this PCR product and the 450-bp product is preferentially synthesized because of its shorter size. The 450-bp product hybridizes to a full-length

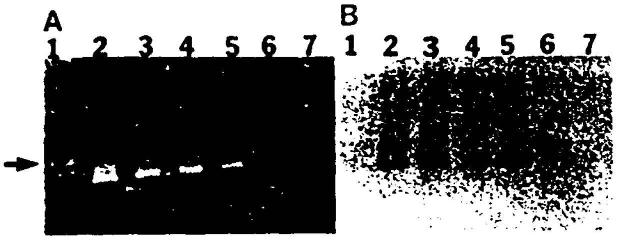

FIG. 2. Sensitivity of PCR assay. Serial 10-fold dilutions of *C. coli* VC167 DNA were subjected to PCR analysis and were run on 2% agarose gels (A) or were hybridized to the internal flagellin probe (B). DNA concentrations were as follows: bacteriophage λ *Hin*dIII marker (the position of the 560-bp fragment is indicated by the arrow) (lanes 1), 62 pg (lanes 2), 6.2 pg (lanes 3), 0.62 pg (lanes 4), 0.062 pg (lanes 5), 0.0062 pg (lanes 6), and 0.62 fg (lanes 7).

flagellin gene probe from plasmid pGK213 (13; data not shown) and to a probe internal to the primer pairs (see below).

The primers were initially evaluated for their ability to amplify a corresponding product from other strains of *C. coli* and from the *C. jejuni* strains. The primers generated the appropriately sized fragment from DNA preparations from 3 other strains of *C. coli* and from 47 strains of *C. jejuni* isolated from a variety of geographical locations (Table 1). Hybridization studies with pGK213 as the probe (13) indicated that the amplified material is flagellin specific (data not shown). As summarized in Table 1, the primers failed to generate a detectable PCR product with DNA from *Campylobacter fetus* subsp. *intermedius* (3 strains), *Campylobacter fetus* subsp. *fetus* (7 strains), *Campylobacter lari* (3 strains), *Campylobacter upsaliensis* (1 strain), *Campylobacter butzleri* (1 strain), *Campylobacter cryaerophila* (or *Arcobacter cryoaerophilus*; 1 strain) (39), *Campylobacter hyointestinalis* (3 strains), *Wolinella recta* (or *Campylobacter recta*; 1 strain) (39), *Helicobacter pylori* (4 strains), *Aeromonas hydrophila* (10 strains), *Aeromonas sobria* (1 strain), *Aeromonas salmonicida* (8 strains), enterotoxigenic *Escherichia coli* (53 strains), *Shigella dysenteriae* (3 strains), *Shigella flexneri* (2 strains), *Shigella sonnei* (8 strains), *Salmonella typhi* (5 strains), *Salmonella enteritidis* (4 strains), *Salmonella typhimurium* (1 strain), *Citrobacter freundii* (1 strain), and *Vibrio cholerae* (13 strains).

Sensitivity of the pg3-pg50 primer pair. In order to determine the sensitivity of the assay, a probe internal to the pg3-pg50 primer pair was isolated. The PCR product from amplification of VC167 DNA was digested with *Bgl*II and *Eco*RI and cloned into pUC18 (Fig. 1). The resulting 273-bp fragment was purified and labeled with digoxigenin. Tenfold serial dilutions of VC167 DNA were subjected to PCR amplification by using the pg3-pg50 primers, and the products were electrophoresed and transferred to a nylon membrane by Southern blotting and hybridized to a digoxigenin-labeled probe. The results indicate that the PCR products generated with as little as 0.062 pg of DNA can be visualized on the agarose gel (Fig. 2A, lane 5) and that hybridization with the internal probe allows detection of as little as 0.0062 pg of DNA (Fig. 2B, lane 6). On the basis of a genome size for *Campylobacter* spp. of 1,700 kbp (4), this corresponds to four or fewer bacteria. In order to determine the sensitivity of the assay for detection of bacteria in stool samples, a normal human stool specimen was divided into 1-g samples and seeded with serial dilutions of VC167 cells. Each 0.5-g sample of seeded stool was extracted by the method of Branstrom et al. (2) into a final volume of 400 μl, and 5 μl of that extracted sample was used in the PCR assay. The results of two separate experiments (data not shown) indicated that by hybridization analysis the assay could detect between 30 and 60 bacterial cells per 5 μl per PCR assay. The gel analysis was again 10-fold less sensitive than hybridization (data not shown).

Detection of *C. coli* in rabbit rectal swabs. In order to evaluate the feasibility of the direct detection of campylobacters in fecal material by PCR, rectal swabs were taken from rabbits which were fed *C. coli* VC167 2 days prior to sampling and from 15 control rabbits which were fed sterile culture broth. Following transport to the laboratory in Cary Blair medium, aliquots of fecal material from each rabbit were resuspended in phosphate-buffered saline as described in Materials and Methods. An aliquot from each sample was plated directly onto campylobacter blood agar, and the plates were incubated microaerobically for 48 h. Another aliquot was processed for PCR by the extraction method of Frankel et al. (10), and the products were dot blotted and hybridized to the nonradioactively labeled internal probe from pBA273. The PCR assay detected *C. coli* in all 15 infected rabbits, although only 12 of 15 rabbits were positive by plating. No campylobacters were detected in the uninfected control rabbits by either PCR or plating.

Detection of *C. jejuni* and *C. coli* in clinical stool specimens. Frozen stool specimens (both culture positive and culture negative for *C. jejuni*) were obtained from field sites in Kuwait and Egypt, as were the *C. jejuni* isolates from the positive stools. Stool samples were processed for PCR by the method of Branstrom et al. (2), and PCR products were electrophoresed and probed with pBA273. Results for selected samples are shown in Fig. 3A and B. Four normal stool samples and four stool samples which were culture positive for *Shigella* spp. were tested; all eight of these samples were negative in the PCR assay (for example, see Fig. 3A and B, lanes 3). Thirteen stool samples which were

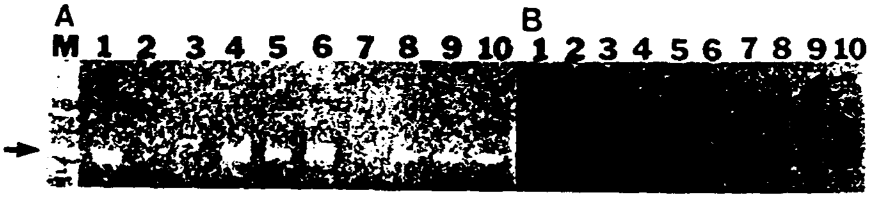

FIG. 3. PCR analysis of representative stool samples. (A) Agarose gel analysis; (B) hybridization analysis. Aliquots of 0.5 g of stools were extracted (3) and resuspended in a final volume of 400 μl, and 5 μl was used per PCR assay. Lane M, 1-kb ladder marker (the arrow indicates the position of the 500-bp fragment); lanes 1, 718; lanes 2, MK52; lanes 3, 553; lanes 4, 797; lanes 5, 630; lanes 6, MK58; lanes 7, 659; lanes 8, MK41; lanes 9, MK19; lanes 10, MK34.

culture positive for *C. jejuni* were examined. Of these 13 stool samples, 9 were positive by PCR, as determined by agarose gel analysis. Representative examples are shown in Fig. 3A and B, lanes 1 and 4 to 10. Sample 659 gave a weak band which did not photograph well, but it was clearly positive by hybridization analysis (Fig. 3B, lane 7). A fourth sample was determined to be positive only after hybridization to the probe (Fig. 3A and B, lanes 2). Three culture-positive stool samples from Egypt were also negative following hybridization (data not shown). The strains which were isolated from two of these stool samples were positive when examined directly by PCR analysis, but the isolate from the third stool sample was negative by the PCR assay.

DISCUSSION

Although a number of DNA probes have been developed for identification of campylobacters (29, 35), no study that used PCR technology to detect these organisms has been published. Results of the present study indicate that the campylobacter flagellin gene can serve as a sensitive and specific target gene for PCR detection of *C. coli* or *C. jejuni*, or both. If the 13 clinical specimens examined in this study are included, a total of 64 strains of *C. jejuni* and *C. coli* were tested by the PCR assay, with an overall sensitivity of 98.5%. The assay failed to detect representative strains of *C. lari*, *C. butzleri*, *C. cryaerophila* (*A. cryoaerophilus* [39]), *C. hyointestinalis*, *C. upsaliensis*, *C. fetus* subsp. *intermedius*, *C. fetus* subsp. *fetus*, as well as *H. pylori*, *W. recta* (or *C. recta*; 39), *E. coli*, *Shigella* spp., *Salmonella* spp., *Vibrio* spp., *Citrobacter* spp., and *Aeromonas* spp. It should be mentioned that the flagellins of both *C. coli* and *C. jejuni* share a high degree of amino acid conservation with certain regions of flagellins of members of the family *Enterobacteriaceae*. For example, the *C. coli* VC167 flagellin gene shows 33.7% sequence identity with the flagellin gene of *S. typhimurium* from amino acid residues 3 to 169 (14). The identity at the nucleotide level, however, fell off considerably because of differences in codon utilization between members of the family *Enterobacteriaceae* and *Campylobacter* spp.

The primer pairs used in this study were specific for *C. jejuni* and *C. coli*, which together constitute the major human pathogens in the genus *Campylobacter*. The lack of reactivity with other highly related *Campylobacter* spp., particularly *C. lari*, was somewhat surprising. Hybridization analysis of the internal probe to other species of campylobacters indicates that there are DNA sequences shared in this region of the flagellin genes of *C. lari* and *C. upsaliensis* (12), suggesting that alternate primer pairs which would allow identification of these other species may be identified. Additionally, sets of primer pairs may even allow for determination of species among certain campylobacters. Such differentiation may be particularly useful, especially in large-scale epidemiological studies. The inability to differentiate *C. jejuni* from *C. coli* by the PCR assay may be problematic in some situations, particularly in developing countries where the proportion of infections with *C. coli* is higher than that in developed countries (36). In the United States, however, *C. coli* infection is relatively rare, with *C. jejuni* representing 99% of reported *Campylobacter* species in a recent study (34), and many clinical laboratories report isolates simply as *Campylobacter* sp. (11). Moreover, the ability to determine the presence of either *C. jejuni* or *C. coli* rapidly and accurately would be useful, particularly in outbreak situations in which large numbers of specimens need to be handled quickly so that appropriate therapy can be instituted (17). We hope that second-generation PCR assays which are capable of distinguishing *C. jejuni* from *C. coli* can be developed. It should also be mentioned that there are very few reliable biochemical assays available for the differentiation of clinical campylobacter isolates (11). Thus, while hydrolysis of hippurate is normally used to distinguish *C. jejuni* from *C. coli*, the assay is not completely reliable (31). Moreover, numerous other *Campylobacter* spp. are now recognized as human pathogens (23) and need to be differentiated from *C. jejuni* and *C. coli*. The PCR assay described here could also potentially be used clinically to distinguish isolates of these other *Campylobacter* spp. from *C. jejuni* or *C. coli*. Indeed, the flagellin-based PCR assay has proven to be useful and reliable at the level of the reference laboratory (19). The assay could also theoretically be adapted to the detection of campylobacters in food, environmental samples, or animal reservoirs such as chickens.

The assay is capable of detecting 0.062 pg of purified DNA (or ≤40 bacterial cells) by gel analysis or 0.0062 pg (or ≤4 bacterial cells) following hybridization of PCR products to a digoxigenin probe. This sensitivity is similar to that seen for other bacterial detection systems (3, 7, 18, 38, 43). However, the ultimate value of PCR detection of enteric pathogens depends on the ability of an investigator to perform the assay directly on stool samples or rectal swabs, or both. The preliminary studies with animal samples described here indicate that the assay is rapid and sensitive for detecting campylobacters in rectal swabs from animals. When a normal human stool sample was seeded with *C. coli* VC167 cells, the assay detected 30 to 60 organisms per 5-μl PCR sample. This is equivalent to approximately 500 to 1,000 organisms per g of stool. However, it is not unlikely that the level of detection may vary among different stool samples or that the sensitivity may vary when alternate extraction protocols are used. Although there have often been difficulties in adapting rapid diagnostic assays in laboratory situations to clinical laboratories, a number of laboratories have had recent success in the direct detection by PCR of other pathogens in human stool samples (2, 5, 32). Our preliminary results with frozen stool specimens from Kuwait and Egypt indicate that the assay is sensitive and specific for the detection of campylobacters in human clinical samples. There were, however, several campylobacter culture-positive stools which were negative by PCR. In all but one case, the cultures of the stool samples were themselves positive by the PCR assay. This suggests either that there were inhibitors in these particular stool samples or that the numbers of organisms were below the limits of detection of the assay, or both. The particular stool samples used in the present study were frozen for up to 1 year prior to the analyses, and this may also have affected the assay. In addition, there is no information available as to the numbers of bacteria cultured from these stools or the course of disease in the patients from whom the stool samples were obtained. Nonetheless, the preliminary human data presented here are promising enough to warrant further evaluation of the PCR assay, and such studies are under way at several test sites.

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